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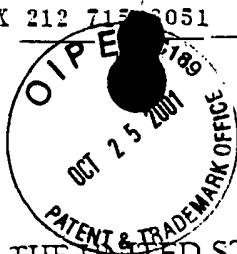
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In The Reissue Of )

Examiner: G. Holliden

U.S. Pat. No. 5,413,774 )

Art Unit: 1616

Reissue Serial No. 09/115,963 )

**DECLARATION OF  
MICHEL SCHNEIDER**
 For: Long-Lasting Aqueous Dispersions )  
 Or Suspensions Of Pressure- )  
 Resistant Gas-filled Microvesicles )  
 And Methods For The Preparation )  
 Thereof. )

I, Michel Schneider, declare as follows:

1. I reside in Troinex, Switzerland.

2. I am a co-inventor of U.S. Patent No. 5,413,774 ("774 patent").

3. A representative sample from the '774 patent was prepared and tested.

Specifically, Example 4 of the parent application to the '774 patent, PCT/EP91/00620 (referred to in the '774 patent col. 4, lines 35-36 as disclosing the preferred microbubbles to be used for the '774 patent) was followed in all respects except C<sub>4</sub>F<sub>10</sub> was used as the gas instead of air. This type of sample is described in the specification of the '774 patent (e.g., col. 4, lines 35-36 discloses this type of phospholipid stabilized microbubble and col. 7, lines 65-68 and original claim 3, which was filed with the application, discloses the C<sub>4</sub>F<sub>10</sub> gas. See also Examples 4-6 of the '774 patent which discloses similar contrast agents). This sample would also fall within the original '774 patent claims directed to stabilized microbubbles, e.g., claims 1 and 4-7 and the corresponding '774 patent reissue claims, e.g., reissue claims 1 and 4-7.

4. Briefly, liposomes (50 mg/ml) were first prepared by the REV method using hydrogenated soy lecithin (NC 95H, Nattermann Chemie GmbH) and dicetylphosphate (Fluka AG) in a 9:1 molar ratio. Two ml of the liposome preparation were added to 8 ml of a 15%

aqueous maltose solution. The resulting solution was frozen at  $-30^{\circ}\text{C}$ , and lyophilised under 0.1 Torr. After complete sublimation of the ice, air was restored in the evacuated chamber. An amount of the resulting powder (0.65g) was introduced in a separate vial, briefly put under vacuum (to eliminate air) and  $\text{C}_4\text{F}_{10}$  (from PCR) was introduced into the vial. Distilled water (5 ml) was finally injected through the stopper and the vial was vigorously shaken for 20 seconds. A milky bubble suspension was obtained. Analysis with a Leica Quantimet showed that the suspension contained between  $10^7$  and  $10^8$  bubbles per ml. The bubbles had a mean diameter of 4-5  $\mu\text{m}$ . The mean diameter of the bubbles in the preparation and the bubble concentration remained essentially constant for at least 30 hours. There was no evidence of coalescence, fusion or ripening. The bubble suspension was very stable.

5. The echographic examination of the heart of a rabbit following the injection of 0.1 ml of the '774 patent preparation showed strong opacification of the right ventricle as well as of the left ventricle. The opacification was very persistent in both ventricles, lasting at least 5 minutes.

6. We have found that the '774 sample discussed above, as well as the other stabilized microbubble and microballoon preparations described in the '774 patent as our invention, are stable for at least weeks or months and they are resistant to fusion, coalescence and ripening, with or without pressure applied. In contrast, a bubble preparation without the phospholipids or polymers which form the '774 patent's stabilized microbubbles or microballoons, i.e., free-gas microbubbles, would not be stable for this length of time and would not be as resistant to fusion, collapse or ripening, whether or not pressure is applied. This is confirmed below with examples from U.S. Patent No. 4,466,442 ("Hilmann" or "'442 patent").

7. A representative sample from the Hilmann patent was prepared by following Example 2 of that patent in all respects. Briefly, 2 ml of an aqueous solution containing 10% by weight Pluronic F-68 was drawn up in a syringe and forcefully injected into 8 ml of an aqueous solution of glucose (5% by weight) in a 25 ml vial. The gas phase in the vial was air. The mixture was vigorously shaken. Foam was formed during agitation. An aliquot was withdrawn and observed under the light microscope.

8. Only a relatively small number of bubbles were detected in the sample and most of them were larger than 10 $\mu$ m. Most of the bubbles burst within seconds. After 1 minute or less almost all of the bubbles had disappeared. After vigorous shaking of the vial, 0.5 ml of this preparation was injected into a rabbit. The heart of the animal was examined by ultrasonography using a 7 MHz probe and an Acuson XP10. Contrast could be detected very transiently (a few seconds) in the right ventricle but no contrast was detectable in the left ventricle.

9. We prepared another sample from the Hilmann patent by following Example 2 but we used egg lecithin (final concentration 10 mg/ml) instead of Pluronic F-68. Upon observation under a light microscope, no bubbles were seen. After vigorous shaking of the vial, 0.5 ml of this preparation was injected into a rabbit as described immediately above. No contrast was detectable in the left ventricle of the rabbit. A few spots of increased contrast could be seen for 1-2 seconds in the right ventricle but there was no complete opacification.

10. Both bubble suspensions prepared according to Hilmann Example 2 show the typical behavior of free-gas microbubbles, i.e., large size, limited stability.

11. My conclusions regarding Hilmann are in fact confirmed by others. In particular, Beller (U.S. Patent No. 5,599,523, attached hereto as Exhibit 1) showed that preparations prepared according to Hilmann (referred to as EP-B-0 077 752 in Beller) were unable to opacify

the left ventricle in the dog. The large size of the bubbles obtained is most probably the cause since our in vitro experiments showed that the bubbles that were observed were generally above 10  $\mu\text{m}$  in diameter. Bubbles of that size would be trapped in the lungs and would be unable to reach the left ventricle.

12. On the other hand, phospholipid-stabilized bubbles prepared according to U.S. Patent No. 5,413,774 using  $\text{C}_4\text{F}_{10}$  (and even air), as shown above, show outstanding stability after formation and no significant change in bubble size for periods of at least 44 hours (in the case of air) and 30 hours (for  $\text{C}_4\text{F}_{10}$ ). See Exhibit 2.

13. The Hilmann patent does not disclose the "stabilized microbubbles" of the '774 patent. The '774 patent's stabilized microbubbles have one or more thin layers of amphiphilic surfactants which surround gas at the gas/liquid interface to stabilize the microbubble. The Hilmann patent teaches the use of viscous suspensions which contain free-gas microbubbles. My experiments confirm that in these suspensions, there is no surfactant boundary surrounding the gas at the gas/liquid interface that stabilizes the bubbles. Thus, the viscosity-raising components and other components of Hilmann cannot provide the "stabilized microbubbles" of the '774 patent. The '774 patent's microballoons with a polymer membrane are also very different from the Hilmann suspensions.

14. The fact that viscosity-raising components alone cannot provide the stabilization of the '774 patent's stabilized microbubbles was disclosed in PCT/EP91/00620 ("the '620 application"). The '620 application provides the preferred stabilized microbubbles for the '774 patent (see '774 patent, col. 4, lines 35-36) and it is a priority application for the '774 patent. The '620 application at page 14 states:

It has also been found that the microbubble suspensions of this invention can be diluted with very little loss in the number of microbubbles to be expected from dilution, i.e. even in the case of high dilution ratios, e.g.  $1/10^2$  to  $1/10^4$ , the microbubble count reduction accurately matches with the dilution ratio. This indicates that the stability of the microbubble depends on the surfactant in lamellar form rather than on the presence of stabilizers or viscosity enhancers like in the prior art. This property is advantageous in regard to imaging test reproducibility as the bubbles are not affected by dilution with blood upon injection in to a patient.

15. The stability of the stabilized microbubbles disclosed in Examples 1-9 of the '620 application and 4-6 of the '774 patent, both in vitro and in vivo, further demonstrates that the stabilized microbubbles of the '774 patent are different from the free gas microbubbles of Hilmann. My experiments described herein demonstrate that the free gas microbubbles of Hilmann are neither stable nor useful.

16. In sum, my experiments show that the bubble suspensions prepared according to Hilmann did not possess the appropriate bubble size (size has to be below  $10\ \mu\text{m}$  in diameter, otherwise the bubbles are trapped in the lungs), absence of coalescence (because when bubble coalesce their size increases and therefore also the tendency to be trapped in the lungs), stability during a reasonable period of time (after formation, the bubble suspension has to be introduced in a syringe then is injected; any bubble suspension should be stable at least for a minute or so), capacity to image the left ventricle (a mandatory feature for any ultrasound contrast agent) and persistence in the blood stream (ideally several minutes are desirable to allow for a proper investigation of the patient). Thus, the Hilmann patent does not disclose clinically useful agents.

17. The substantially improved results in stability (both in vitro and in vivo) and the ability to image the left ventricle achieved by the stabilized microbubbles of the '774 patent were unexpected and I am not aware of any evidence to suggest that these substantially improved results would have been expected.

All statements made of my own knowledge are true and all statements made on information and belief are believed to be true. I make this declaration understanding that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the applications or any patent issuing thereon.

Respectfully submitted,



Michel Schneider, Ph.D.

Dated: March 24<sup>th</sup>, 2000

# United States Patent

Beller et al.

[11] Patent Number: 5,599,523

[45] Date of Patent: Feb. 4, 1997

[54] ECHO CONTRAST AGENT

[75] Inventors: Klaus-Dieter Beller; Rudolf Linder,  
both of Konstanz, Germany

[73] Assignee: BYK Gulden Lomberg Chemische  
Fabrik GmbH, Konstanz, Germany

[21] Appl. No.: 347,206

[22] Filed: Nov. 22, 1994

## Related U.S. Application Data

[63] Continuation of Ser. No. 78,189, filed as PCT/EP92/00012,  
Jan. 4, 1992 published as WO92/11873, Jul. 23, 1992.

## [30] Foreign Application Priority Data

Jan. 9, 1991 [DE] Germany ..... 41 00 470.1

[51] Int. Cl.<sup>6</sup> ..... A61K 49/00

[52] U.S. Cl. .... 424/9.52; 424/9.5; 424/9.51

[58] Field of Search ..... 424/9, 450, 9.5,  
424/9.51, 9.52; 128/662.02

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## [57] ABSTRACT

An aqueous preparation for receiving and stabilising micro gas bubbles for use as echo contrast media containing polyoxyethylene/polyoxypropylene polymers and negatively charged phospholipids, which is suitable for demonstration of the left ventricle, is indicated.

24 Claims, 1 Drawing Sheet

FIG. 1

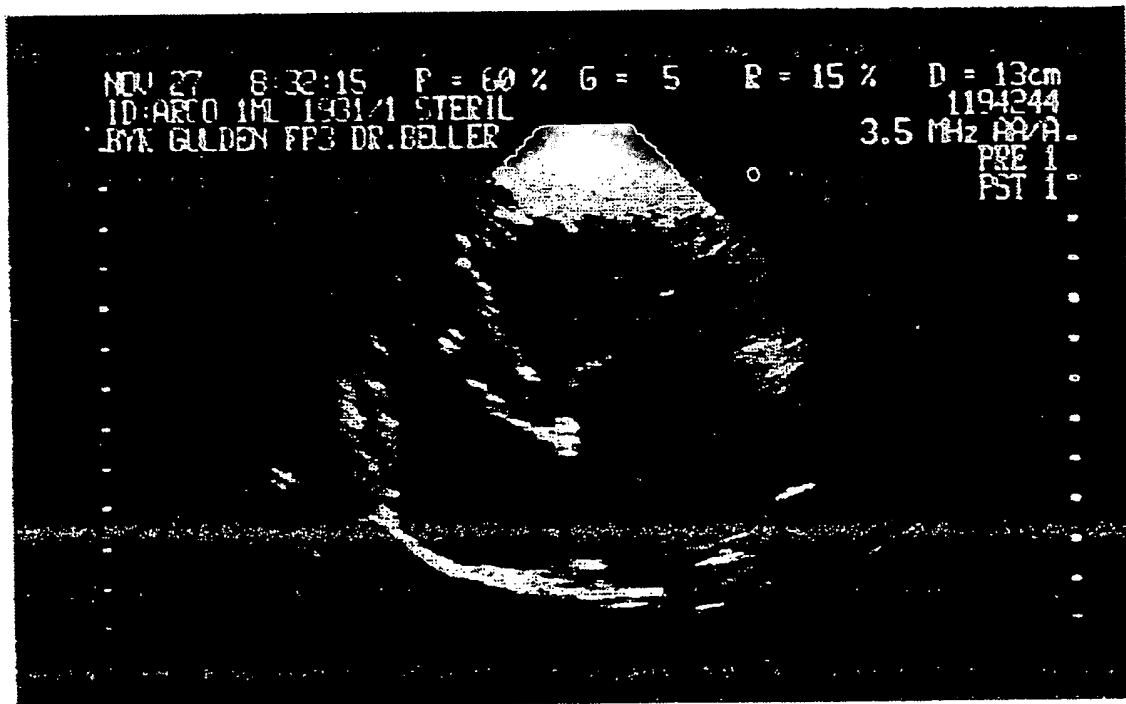
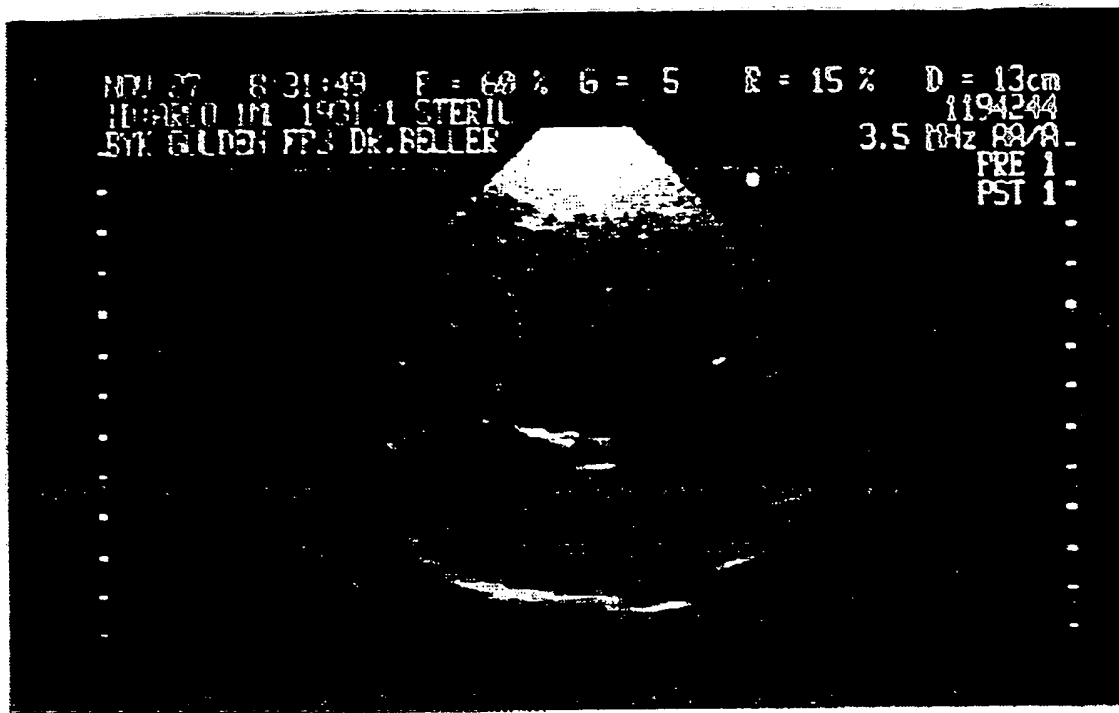


FIG. 2

## ECHO CONTRAST AGENT

This application is a continuation of application Ser. No. 08/078,189, filed Jun. 21, 1993, now abandoned, which is a 371 of PCT/EP92/00012, filed Jan. 4, 1992.

## FIELD OF THE INVENTION

The invention relates to an aqueous preparation for receiving and stabilising micro gas bubbles for use as echo contrast media.

## PRIOR ART

Since ultrasound is highly reflected by gas bubbles suspended in liquids, there was an early proposal to use aqueous preparations which contain stabilised micro gas bubbles as contrast media for ultrasonic diagnosis. Micro gas bubbles can be stabilised in aqueous preparations by reducing the surface tension, that is to say by adding suitable surfactants.

It is indicated in EP-B-0 077 752 that aqueous solutions which contain a surfactant or a mixture of surfactants and, in addition, a viscosity-increasing substance have advantageous contrast-generating properties. Indicated as suitable surfactants are, inter alia, non-ionic lecithins and lecithin fractions, and polyoxyethylene/polyoxypropylene polymers. The preparations indicated in the six preparation examples of EP-B-0 077 752 each contain as surfactant a polyoxyethylene/polyoxypropylene polymer and as viscosity-increasing substance glucose or dextran or the polyoxyethylene/polyoxypropylene polymer itself. Repetition of the preparation examples has shown that the contrast-generating action is unsatisfactory. Thus, the preparations of EP-B-0 077 752 are unsuitable for demonstrations of the left ventricle.

It has now been found, surprisingly, that aqueous preparations which, besides polyoxyethylene/polyoxypropylene polymers, contain negatively charged phospholipids are outstandingly suitable for receiving and stabilising micro gas bubbles.

## SUMMARY OF THE INVENTION

The invention therefore relates to aqueous preparations for receiving and stabilising micro gas bubbles for use as echo contrast media containing polyoxyethylene/polyoxypropylene polymers and negatively charged phospholipids.

Further subject-matter is evident from the claims.

Preferred polyoxyethylene/polyoxypropylene polymers are those with an average molecular weight of 8,350 to 14,000. Polyoxyethylene/polyoxypropylene polymers are also called poloxamers and are commercially available, for example, under the proprietary name Pluronic® (Wyandotte Chemicals Corp.). The preparations according to the invention contain 0.1 to 10%, preferably 1 to 5%, of polyoxyethylene/polyoxypropylene polymers. The content of negatively charged phospholipids is from 0.01 to 5%, preferably 0.5 to 2%. Percentage data in each case relate to weight/volume.

Suitable negatively charged phospholipids are phosphatidylglycerols, phosphatidylinositols, phosphatidylethanolamines and phosphatidylserines and the lyso forms thereof. By lyso forms of the negatively charged phospholipids are meant negatively charged phospholipids which contain only one acyl radical. Lyso forms of the negatively charged phospholipids in which the acyl group is bonded to the oxygen of the carbon atom 1 of the glycerol molecule are

preferred. Particularly preferred negatively charged phospholipids are dipalmitoylphosphatidylglycerol (DPPG) and distearoylphosphatidylglycerol (DSPG), and distearoylphosphatidylglycerol (DSPG) is very particularly preferred.

The preparations according to the invention are distinguished from those of the prior art in that echo contrast media containing micro gas bubbles can be produced with little mechanical effort and, because of their great stability, generate a long-lasting contrast and moreover are outstandingly suitable for demonstration of the left ventricle. It should be particularly emphasised that the preparations according to the invention are excellently suitable for the demonstration of internal surface structures because the micro gas bubbles apparently adhere well to surfaces and thus generate informative contrast even after the micro gas bubbles which are located in the lumen of vessels have been flushed out. This makes it possible, for example, to demonstrate more clearly the dynamics of the heart even after the contrast medium has been washed out.

The preparation of the preparations according to the invention is not difficult and can take place by introducing the individual components together or successively into water and dissolving, if necessary with heating and stirring. Sterilisation is also possible if required, for example by heat sterilisation.

Glycerol, mannitol and ammonium salts of amino acids, preferably glycine, have proved particularly well suited for adjusting the isotonicity of the preparations according to the invention.

The micro gas bubbles are generated in a manner known per se and expediently only shortly before administration to the patients to be investigated. If, for example, the preparation according to the invention is provided in a vial, the solution can be drawn up together with the required amount of air into a conventional syringe and injected again into the vial through a narrow needle with the highest possible pressure. If necessary, the drawing up and expulsion from the syringe is repeated several times. It is also possible as an alternative to force the preparations according to the invention backwards and forwards between two syringes via a connector with a narrow cross-section or a mixing chamber inserted between the two syringes. The latter method leads to particularly informative ultrasonic images with, at the same time, a further increase in productivity.

Suitable gases for generating the micro gas bubbles are all physiologically tolerated gases. The preparations according to the invention are converted into a foam with 0.01 to 0.1, preferably with 0.04 to 0.06, ml of gas per 1 ml. They are preferably administered intravenously after generation of the micro gas bubbles. Depending on the purpose for which the preparations according to the invention are used, 1 to 20 ml, preferably 2 to 8 ml, and particularly preferably 5 ml are administered.

It should be particularly emphasised that lower doses of the preparations according to the invention are possible because of their increased productivity compared with the prior art.

## EXAMPLES

1. 3.0 g of polyoxyethylene/polyoxypropylene polymer with an average molecular weight of 8,400 (Pluronic®F68), 1.0 g of dipalmitoylphosphatidylglycerol (DPPG) and 3.6 g of glycerol are introduced into 80 ml of water. The mixture is heated to about 80° C. and stirred until complete disso-

lution has taken place. After cooling, the volume is made up to 100 ml with distilled water.

2. The process is carried out as in Example 1 with the difference that 1.0 g of soya phosphatidylglycerol (supplied by Lucas Meyer, Hamburg) is used in place of DPPG.

3. 1.1 g of glycine are introduced into 80 ml of water. A pH of 6 to 7 is adjusted with dilute ammonia. 3.0 g of polyoxyethylene/polyoxypropylene polymer with an average molecular weight of 8,400 (Pluronic®F68) and 1.0 g of DPPG are added to the solution. The mixture is heated to about 80° C. and stirred until complete dissolution has taken place. After cooling, the volume is made up to 100 ml with distilled water.

4. The process is carried out as in Example 3 with the difference that 1.0 g of soya phosphatidylglycerol (supplied by Lucas Meyer) is used in place of DPPG.

5. 4.0 g of polyoxyethylene/polyoxypropylene polymer (Poloxamer 188, Pluronic®F68), 1.0 g of distearoylphosphatidylglycerol and 5.4 g of mannitol are introduced into 80 ml of water. The mixture is heated to about 80° C. and stirred until dissolution is complete. After cooling, the volume is made up to 100 ml with distilled water.

#### COMPARATIVE EXPERIMENTS

The investigations were carried out on conscious male beagle dogs (18.2–30.5 kg body weight). The dogs received in each case 5 ml, administered i.v., of the contrast medium preparations described below:

A: An infusion solution, containing 35 g of crosslinked polypeptides per 1,000 ml, for plasma replacement (Haemaccel® supplied by Behringwerke)

B: Echovist® (echo contrast medium supplied by Schering)

C: An aqueous solution containing 4% by weight poloxamer 188 (Pluronic®F68) and 4% by weight glucose (Example 1 in EP 0 077 752)

D: An aqueous solution containing 2% by weight poloxamer and 4% by weight glucose (Example 2 in EP 0 077 752)

E: An aqueous solution containing 1% by weight poloxamer and 4% by weight glucose (Example 3 in EP 0 077 752)

F: Preparation according to the invention from Example 5

Solutions A, C, D, E and F are drawn up without air into a first syringe. This syringe is then connected by the free end to a mixing chamber which contains 0.18 ml of air and is firmly connected to a second syringe. Immediately before administration, the solutions are pumped out of the first syringe through the mixing chamber into the second syringe and back again five times.

The commercially available contrast medium B is prepared as instructed in the pack insert.

The echocardiographic ultrasonic scans were carried out with a Sonoscope 4 ultrasonic instrument with mechanical head at 3.5 MHz. The videotapes of the resulting ultrasonic images were evaluated for the intensity of contrast by densitometry. The densitometer used (Gretag D182) determines the changes in the brightness in 100 steps in the range from 0.00 to 2.50 density units. The calibration is carried out using the DIN 16536 calibration card (calibration reference) provided by the manufacturer, where the lightest white is assigned the value 1.64 and the darkest black is assigned the value 0.00. The average of four individual determinations on

an area of 1 cm×1 cm gives the value for the administered preparation for each animal.

The results obtained are shown in the table which follows.

S ml	right ventricle			left ventricle		
	Contrast	Intensity		Contrast	Intensity	
		max	10 sec		max	10 sec
A	yes	1.18	0.86	no	0.00	0.00
B	yes	1.09	0.65	no	0.00	0.00
C	yes	1.20	0.78	no	0.00	0.00
D	yes	1.23	0.87	no	0.00	0.00
E	yes	1.22	0.93	no	0.00	0.00
F	yes	1.19	0.82	yes	0.78	0.72

#### Intensity in Density Units (DU)

It is evident from the results that the echo contrast media according to the invention enter the lungs, in contrast to the echo contrast media of the prior art, and are therefore excellently suited for diagnosis in the left ventricle. The utilisability of ultrasonic imaging in cardiac diagnosis is considerably extended by the echo contrast media according to the invention.

In addition, it has been found that the micro bubbles of the echo contrast media according to the invention apparently have a considerable affinity for the internal surfaces of vessels and cavities in the body. The consequence of this is that the outlines of vessels and cavities are demonstrated much better and therefore more informatively than was possible with contrast media of the prior art. It is particularly advantageous in this connection that this great improvement in the demonstration of the surfaces of vessels and cavities even persists when the lumen of the vessel or cavity is already free of echo contrast medium. This surprising contrast of surfaces can be utilised, for example, for observation of the endocardium.

FIGS. 1 and 2 depict the result of an experiment to demonstrate this novel contrast of surface structures.

FIG. 1 shows the echocardiographic image of the endocardium of a conscious beagle dog in the so-called four-chamber view immediately before appearance of the first contrast after administration of 1 ml of echo contrast medium from Example 1.

FIG. 2 shows the endocardium of the animal after the echo contrast medium has already been washed out of the heart again.

It is evident from comparison of the two figures that an unexpected marking of the endocardium, which signifies a large gain in information for diagnostic purposes, is possible with echo contrast media according to the invention.

We claim:

1. An aqueous preparation useful for receiving and stabilising micro gas bubbles for use as echo contrast media and containing polyoxyethylene/polyoxypropylene polymer and anionic phospholipid.

2. An aqueous preparation of claim 1 which comprises, as sole essential components, water, polyoxyethylene/polyoxypropylene polymer and anionic phospholipid, optionally in combination with isotonicity adjusting component means.

3. A preparation according to claim 2, wherein the polyoxyethylene/polyoxypropylene polymer comprises from 0.1 to 10% (weight/volume).

4. A preparation according to claim 3, wherein the poly-

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oxyethylene/polyoxypropylene polymer comprises from 1 to 5% (weight/volume).

5. A preparation according to claim 1, containing phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine or phosphatidylserine as the anionic phospholipid.

6. A preparation according to claim 5, containing distearoylphosphatidylglycerol as the anionic phospholipid.

7. A preparation according to claim 1, containing the anionic phospholipid in an amount of from 0.01 to 5% (weight/volume).

8. A preparation according to claim 1, containing 3% (weight/volume) of polyoxyethylene/polyoxypropylene polymer with an average molecular weight of 8,400 and 1% (weight/volume) of distearoylphosphatidylglycerol.

9. A preparation according to claim 1, wherein the anionic phospholipid is present as lyso form.

10. A process for the preparation of an aqueous preparation for receiving and stabilising micro bubbles for use as echo contrast media, which comprises dissolving in water polyoxyethylene/polyoxypropylene polymer together with a anionic phospholipid and customary auxiliaries for achieving isotonicity.

11. In an aqueous echo contrast medium containing polyoxyethylene/polyoxypropylene polymer, the improvement wherein the polymer is in admixture with anionic phospholipid.

12. An aqueous preparation of claim 2 which consists essentially of polyoxyethylene/polyoxypropylene polymer and anionic phospholipid dissolved in water.

13. An aqueous preparation of claim 2 useful for receiving and stabilizing micro gas bubbles for use as echo contrast medium and containing polyoxyethylene/polyoxypropylene polymer and anionic phospholipid;

the polymer having an average molecular weight of from 8,350 to 14,000; and

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the anionic phospholipid being a member selected from the group consisting of a phosphatidylglycerol, a phosphatidylinositol, a phosphatidylethanolamine and a phosphatidylserine.

14. An aqueous preparation according to claim 13 containing from 0.1 to 10 percent (weight/volume) of the polyoxyethylene/polyoxypropylene polymer.

15. An aqueous preparation according to claim 14 containing from 0.01 to 5 percent (weight/volume) of the anionic phospholipid.

16. An aqueous preparation according to claim 15 wherein the anionic phospholipid is a phosphatidylglycerol.

17. An aqueous preparation according to claim 15 wherein the anionic phospholipid is a phosphatidylinositol.

18. An aqueous preparation according to claim 15 wherein the anionic phospholipid is a phosphatidylethanolamine.

19. An aqueous preparation according to claim 15 wherein the anionic phospholipid is a phosphatidylserine.

20. An aqueous preparation of claim 1 which is suitable for demonstration of the left ventricle.

21. A preparation according to claim 1, wherein the polyoxyethylene/polyoxypropylene polymer has an average molecular weight of from 8,350 to 14,000.

22. An echo contrast medium which is an aqueous preparation of claim 1 in intimate admixture with stabilized micro gas bubbles.

23. An echo contrast medium which is an aqueous preparation of claim 21 in intimate admixture with stabilized micro gas bubbles.

24. An echo contrast medium of claim 22, having great stability and being produced with little mechanical effort.

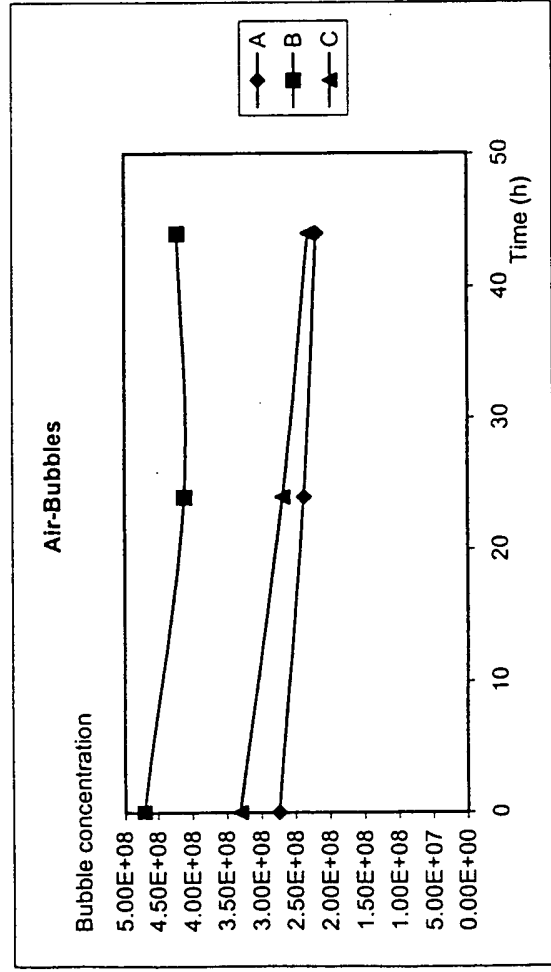
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# Stability of NC95H/DCP (9/1) / Maltose native microbubbles ( 3 batches A - B - C )

## AIR-filled Microbubbles

AIR Time (h)	Bubble Concentration (Nb/ml)			Mean
	A	B	C	
0	2.74E+08	4.71E+08	3.31E+08	3.59E+08
24	2.37E+08	4.11E+08	2.68E+08	3.05E+08 = 85.1%
44	2.19E+08	4.21E+08	2.30E+08	2.90E+08 = 80.9%

AIR Time (h)	Number mean Diameter (µm)		
	A	B	C
0	2.5	2.6	2.4
24	2.4	2.5	2.3
44	2.4	2.6	2.3



## C4F10-filled Microbubbles

C4F10 Time (h)	Bubble Concentration (Nb/ml)			Mean
	A	B	C	
0	2.20E+07	2.41E+07	1.25E+07	1.95E+07
5	1.84E+07	1.96E+07	1.49E+07	1.76E+07 = 90.3%
30	1.54E+07	1.66E+07	1.14E+07	1.45E+07 = 74.1%

C4F10 Time (h)	Number mean Diameter (µm)		
	A	B	C
0	4.0	4.4	5.0
5	4.0	4.8	4.9
30	4.5	4.8	5.7

